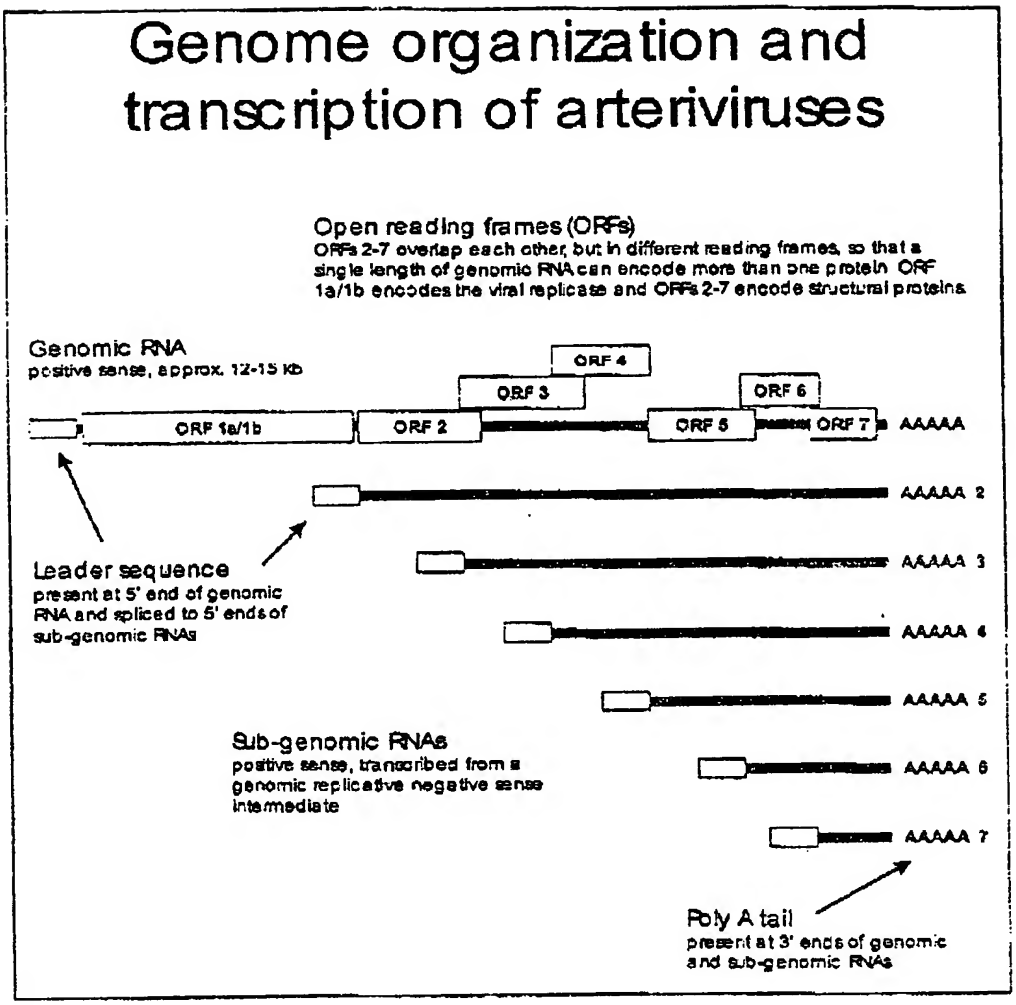


Fig. 1:

Schematic diagram of the genomic organization and transcriptional strategy of the family *Arteriviridae*.

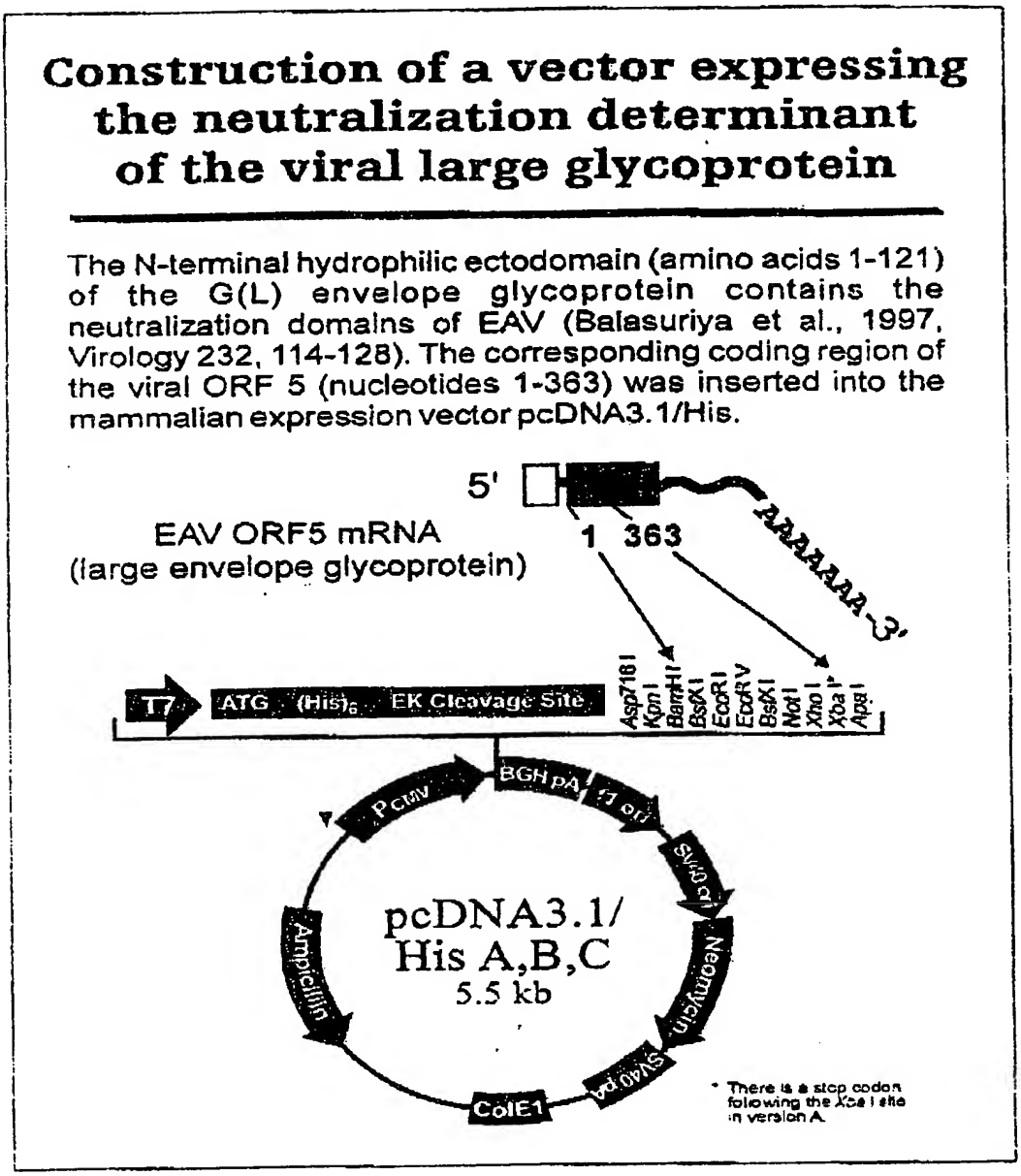
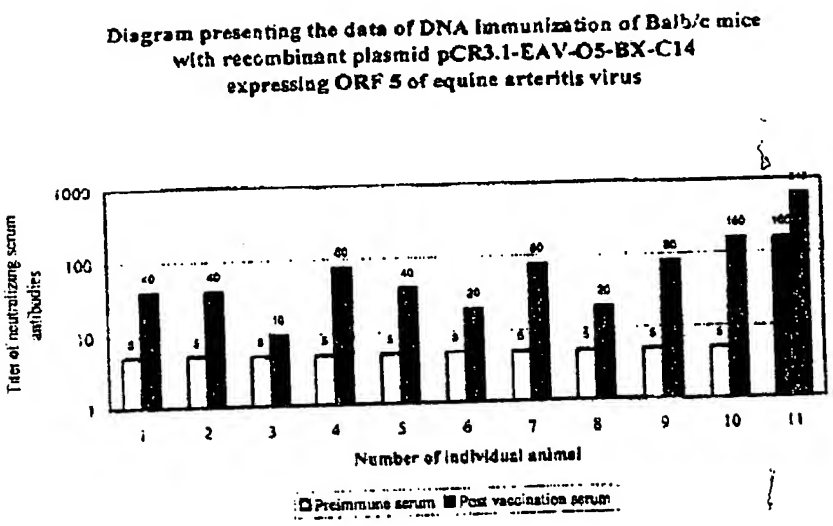


Fig. 2:

Schematic diagram of the strategy used for molecular cloning of neutralizing domain of equine arteritis virus (EAV). A part of the cDNA of viral ORF5 expressing the N-terminal hydrophilic ectodomain of the EAV envelope large glycoprotein was inserted into the corresponding sites of mammalian expression vector pcDNA3.1.

A



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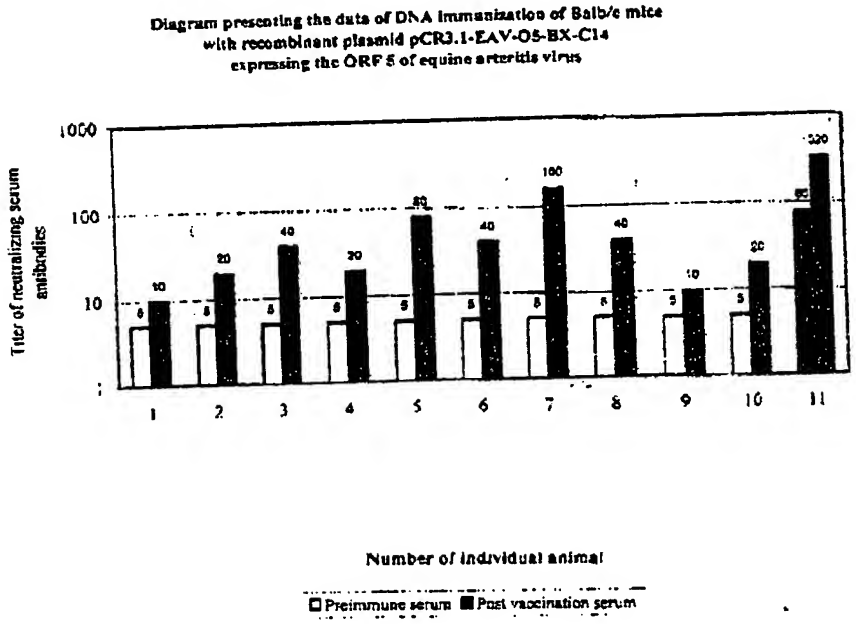


Fig. 3:

The results of neutralization tests obtained by the analysis of the sera of the individual Balb/c mice that were inoculated in two independent experiments (A and B) with the DNA of recombinant plasmid pCR3.1-EAV-O5-BX-C14 harboring and expressing ORF 5 of equine arteritis virus (EAV). The individual animals are indicated with number 1 to 10. The white and black columns represent the data of pre and post DNA vaccinated animals, respectively. The column 11 (black color) served as internal positive control and indicates the average of maximum and minimum neutralizing titer obtained from the serum of a New Zealand white rabbit that was immunized with inactivated EAV.

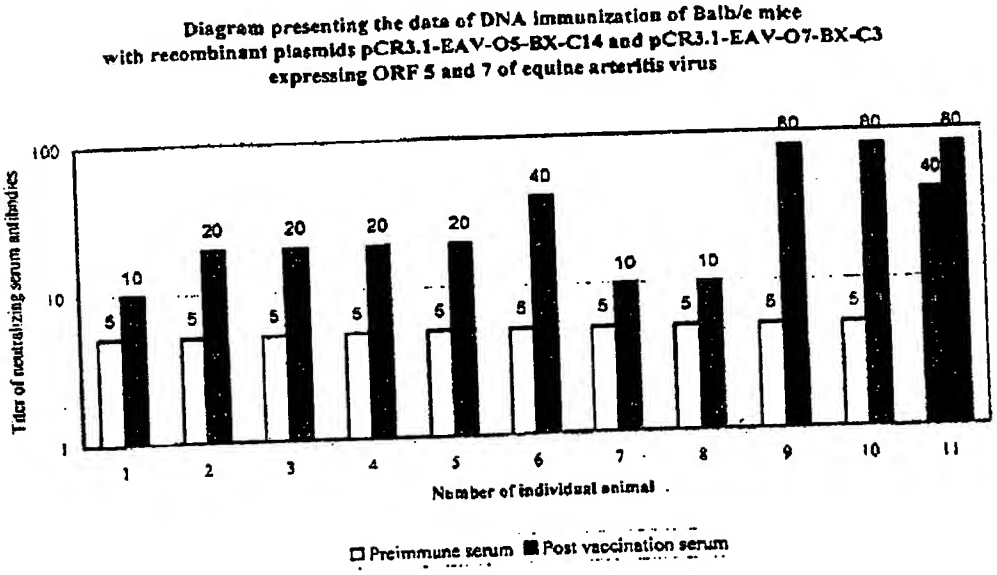


Fig. 4:

The results of neutralization test obtained by the analysis of the sera of the individual Balb/c mice that were inoculated with the DNA of recombinant plasmids pCR3.1-EAV-O5-BX-C14 and pCR3.1-EAV-O7-BX-C3 harboring and expressing ORFs 5 and 7 of equine arteritis virus (EAV). The individual animals are indicated with number 1 to 10. The white and black columns represent the data of pre and post DNA vaccinated animals, respectively. The column 11 (black color) served as internal positive control and indicates the average of maximum and minimum neutralizing titer obtained from the serum of a New Zealand white rabbit that was immunized with inactivated EAV.

Diagram presenting the data of DNA immunization of Balb/c mice with recombinant plasmids pDP-EAV-O7-BgS-C2 and pDP-EAV-O5-BgS-C1 expressing ORF 7 and 5 of Equine arteritis virus, as well as pWS2ms expressing IL-2 gene

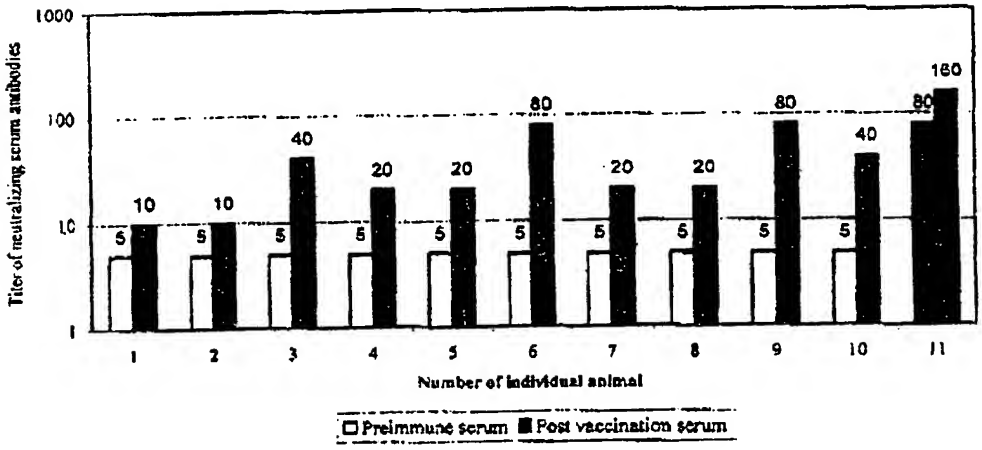


Fig. 5:

The results of neutralization test obtained by the analysis of the sera of the individual Balb/c mice that were inoculated with the DNA of recombinant plasmids pDP-EAV-O5-BsS-C2 and pDP-EAV-O7-BsS-C1 harboring and expressing ORFs 5 and 7 of equine arteritis virus (EAV). The recombinant plasmid pWS2ms expressing mouse IL2 gene was administered as immune modulating factor. The individual animals are indicatesd with number 1 to 10. The white and black columns represent the data of pre and post DNA vaccinated animals, respectively. The column 11 (black color) served as internal positive control and indicates the average of maximum and minimum neutralizing titer obtained from the serum of a New Zealand white rabbit that was immunized with inactivated EAV.

Diagram presenting the data of DNA immunization of Balb/c mice with recombinant plasmids pCR3.1-EAV-O5-BX-C14 and pCR3.1-EAV-O6-BE-C4 expressing ORF 5 and 6 of equine arteritis virus

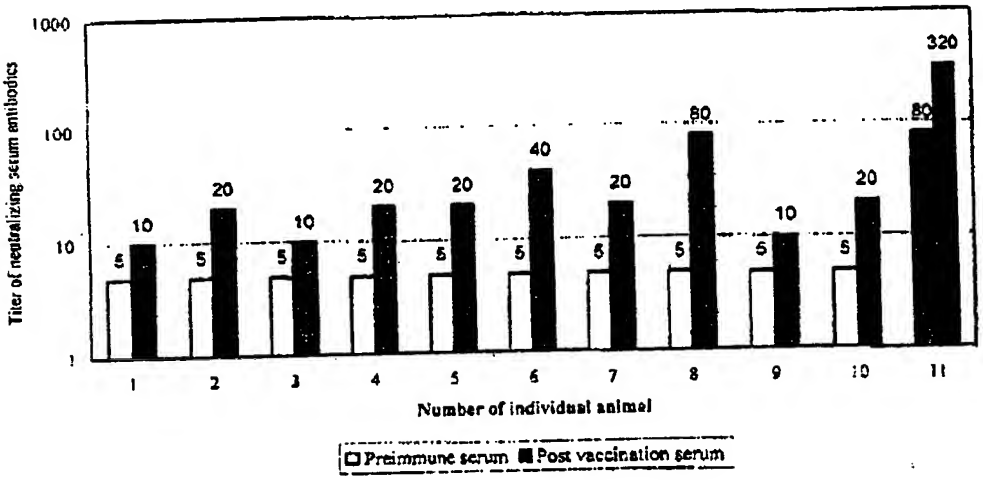


Fig. 6:
The results of neutralization test obtained by the analysis of the sera of the individual Balb/c mice that were inoculated with the DNA of recombinant plasmids pCR3.1-EAV-O5-BX-C14 and pCR3.1-EAV-O6-BE-C4 harboring and expressing ORFs 5 and 6 of equine arteritis virus (EAV). The individual animals are indicated with number 1 to 10. The white and black columns represent the data of pre and post DNA vaccinated animals, respectively. The column 11 (black color) served as internal positive control and indicates the average of maximum and minimum neutralizing titer obtained from the serum of a New Zealand white rabbit that was immunized with inactivated EAV.

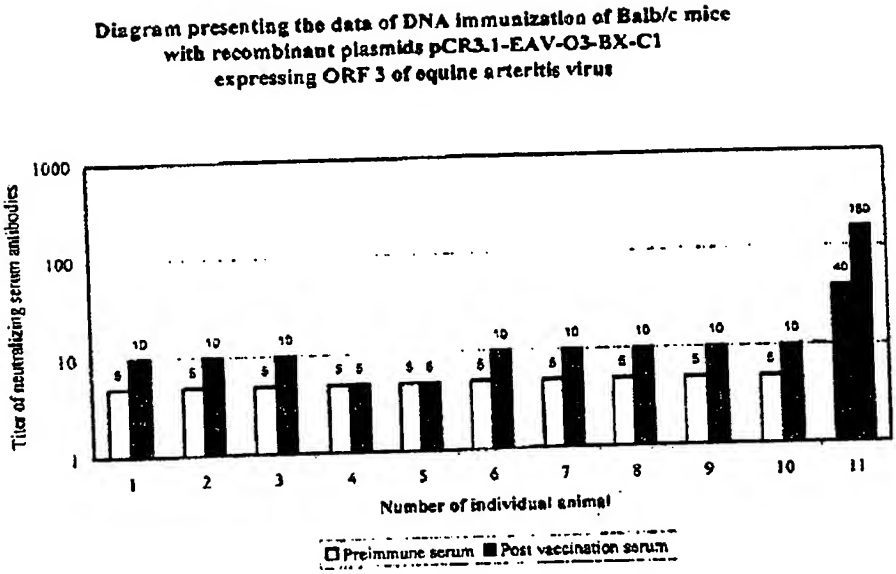


Fig. 7:

The results of neutralization test obtained by the analysis of the sera of the individual Balb/c mice that were inoculated with the DNA of recombinant plasmid pCR3.1-EAV-O4-BX-C3 harboring and expressing ORF 4 of equine arteritis virus (EAV). The individual animals are indicated with number 1 to 10. The white and black columns represent the data of pre and post DNA vaccinated animals, respectively. The column 11 (black color) served as internal positive control and indicates the average of maximum and minimum neutralizing titer obtained from the serum of a New Zealand white rabbit that was immunized with inactivated EAV.

Diagram presenting the data of DNA immunization of Balb/c mice with recombinant plasmids pCR3.1-EAV-O4-BX-C3 expressing ORF 4 of equine arteritis virus

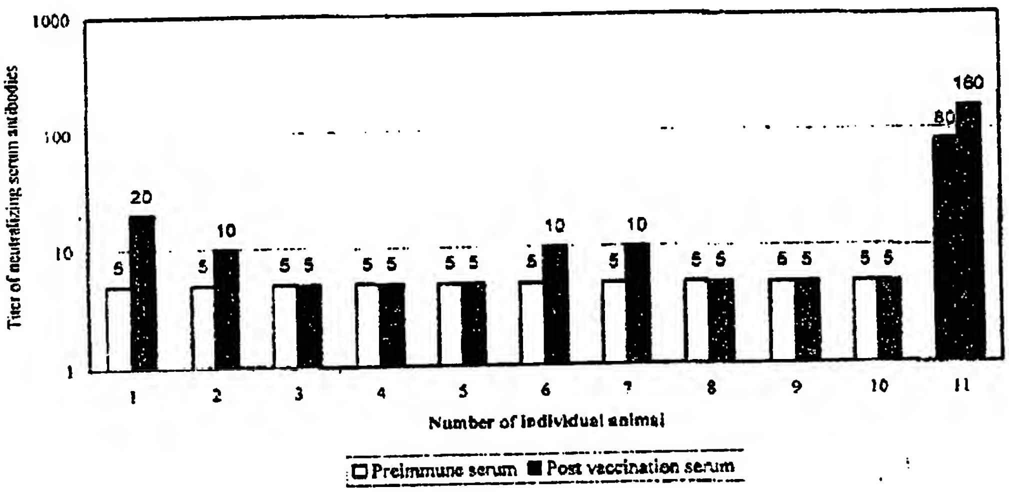


Fig. 8:

The results of neutralization test obtained by the analysis of the sera of the individual Balb/c mice that were inoculated with the DNA of recombinant plasmid pCR3.1-EAV-O4-BE-C3 harboring and expressing ORF 4 of equine arteritis virus (EAV). The individual animals are indicated with number 1 to 10. The white and black columns represent the data of pre and post DNA vaccinated animals, respectively. The column 11 (black color) served as internal positive control and indicates the average of maximum and minimum neutralizing titer obtained from the serum of a New Zealand white rabbit that was immunized with inactivated EAV.

Diagram presenting the data of DNA immunization of Balb/c mice with recombinant plasmid pC3.1-EAV-O5-del-121 expressing the amino terminus (aa 1-121) of ORF 5 of equine arteritis virus

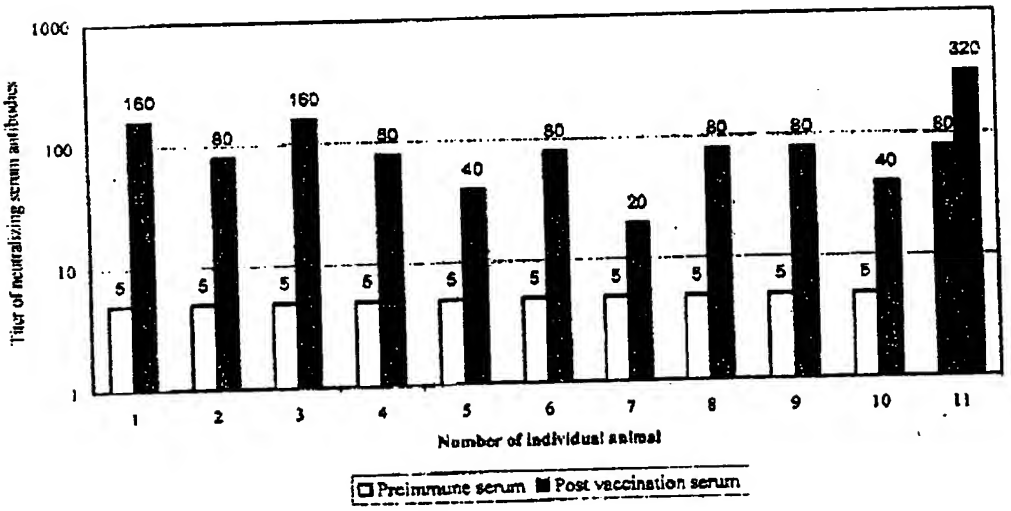


Fig. 9:

The results of neutralization tests obtained by the analysis of the sera of the individual Balb/c mice that were inoculated with the DNA of recombinant plasmid pCR31-EAV-O5-del-121 harboring and expressing the N-terminal hydrophilic ectodomain of G_L envelope glycoprotein (amino acid residue 1-121 of ORF 5) of equine arteritis virus (EAV). The individual animals are indicated with number 1 to 10. The white and black columns represent the data of pre and post DNA vaccinated animals, respectively. The column 11 (black color) served as internal positive control and

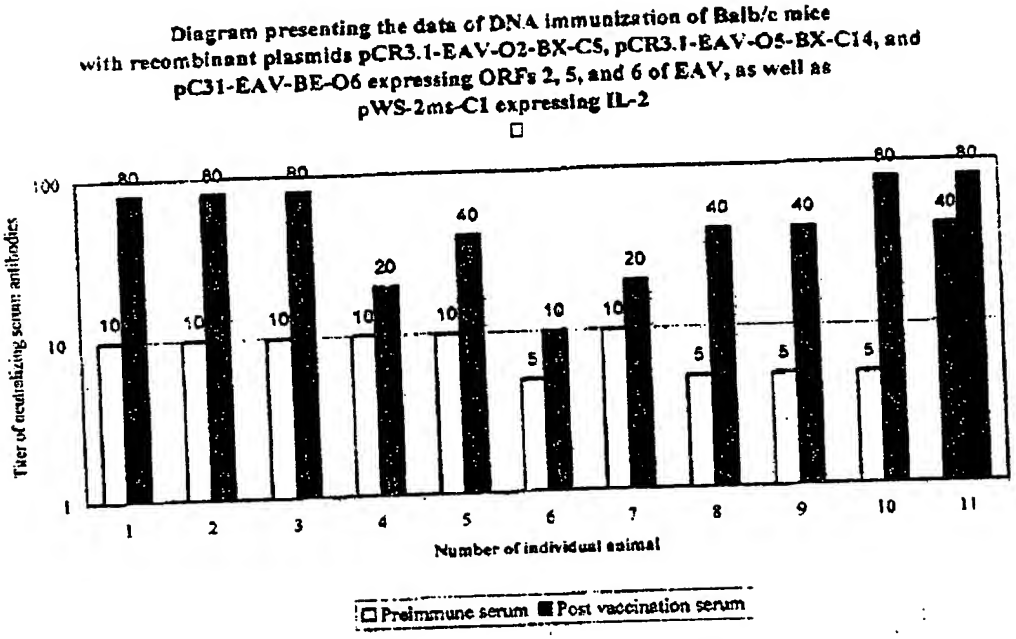


Fig. 10:

The results of neutralization test obtained by the analysis of the sera of the individual Balb/c mice that were inoculated with the DNA of recombinant plasmids pCR3.1-EAV-O2-BX-C5, pCR3.1-EAV-O5-BX-C14, and pCR3.1-EAV-O6-BE-C4 harboring and expressing ORFs 2 (small glycoprotein), 5 (large envelope glycoprotein), and 6 (membrane protein), of equine arteritis virus (EAV). The recombinant plasmid pWS2ms (expressing mouse IL2 gene) was administered as immune modulating factor. The individual animals are indicated with number 1 to 10. The white and black columns represent the data of pre and post DNA vaccinated animals, respectively. The column 11 (black color) served as internal positive control and indicates the average of maximum and minimum neutralizing titer obtained from the serum of a New Zealand white rabbit that was immunized with inactivated EAV.

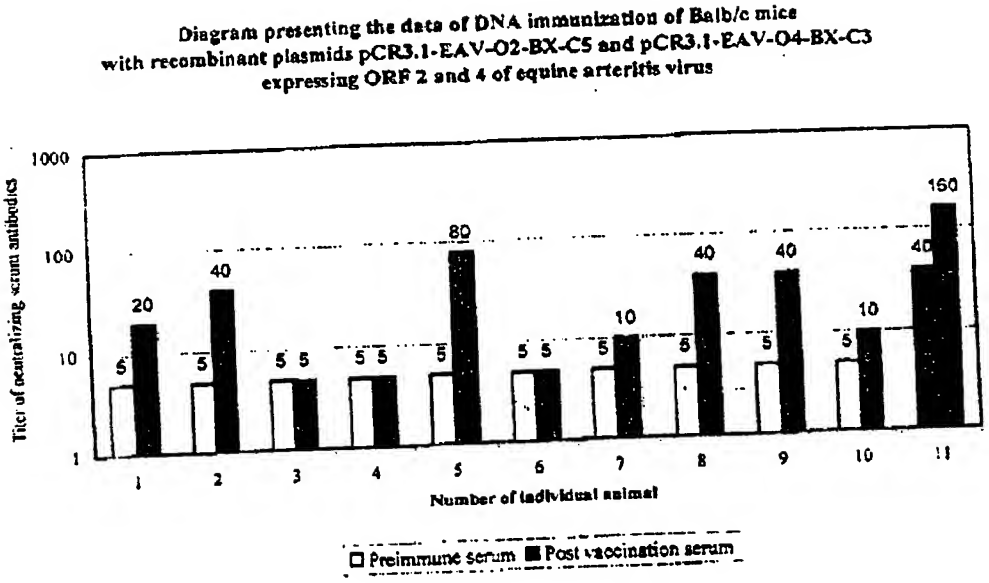


Fig. 11:

The results of neutralization test obtained by the analysis of the sera of the individual Balb/c mice that were inoculated with the DNA of recombinant plasmids pCR3.1-EAV-O2-BX-C5 and pCR3.1-EAV-O4-BX-C3 harboring and expressing ORFs 2 and 4 of equine arteritis virus (EAV). The individual animals are indicated with number 1 to 10. The white and black columns represent the data of pre and post DNA vaccinated animals, respectively. The column 11 (black color) served as internal positive control and indicates the average of maximum and minimum neutralizing titer obtained from the serum of a New Zealand white rabbit that was immunized with inactivated EAV.

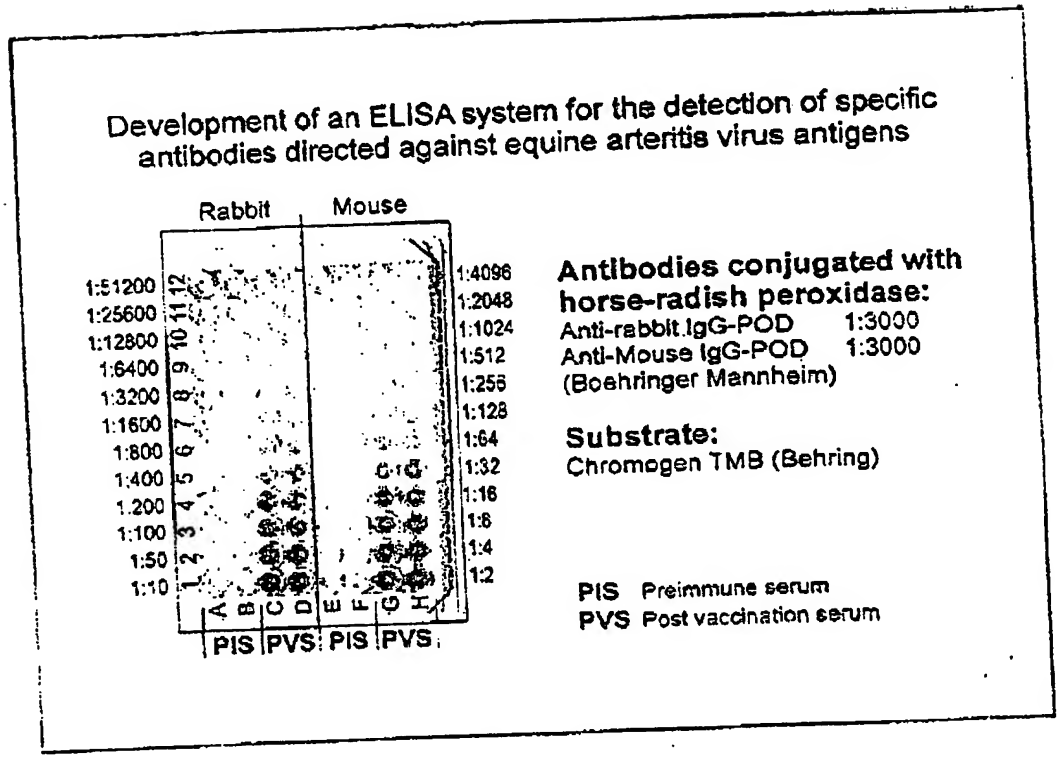


Fig. 12:

An example of the results obtained by enzyme linked immunosorbent assay (ELISA) for the detection of EAV specific antibodies. Polysorp F8 microtiter plates were coated with EAV Protein (EAV+ Host (RK13)) at a concentration of 2 $\mu g \times ml^{-1}$ in PBS (+ 0.05% N_3Na) over night at room temperature (for detail see session material and methods). The assay was stopped after 30 min by the addition of 50 µl/well stopping solution POD and read according to standard procedures at 450 nm on an automatic ELISA reader (MR5000, DYNATECH, Denkendorf, Deutschland). Photograph was taken prior to the analysis of the assay by 450 nm by ELISA reader.

Establishing of a lymphoproliferation assay for detection of cellular immune response

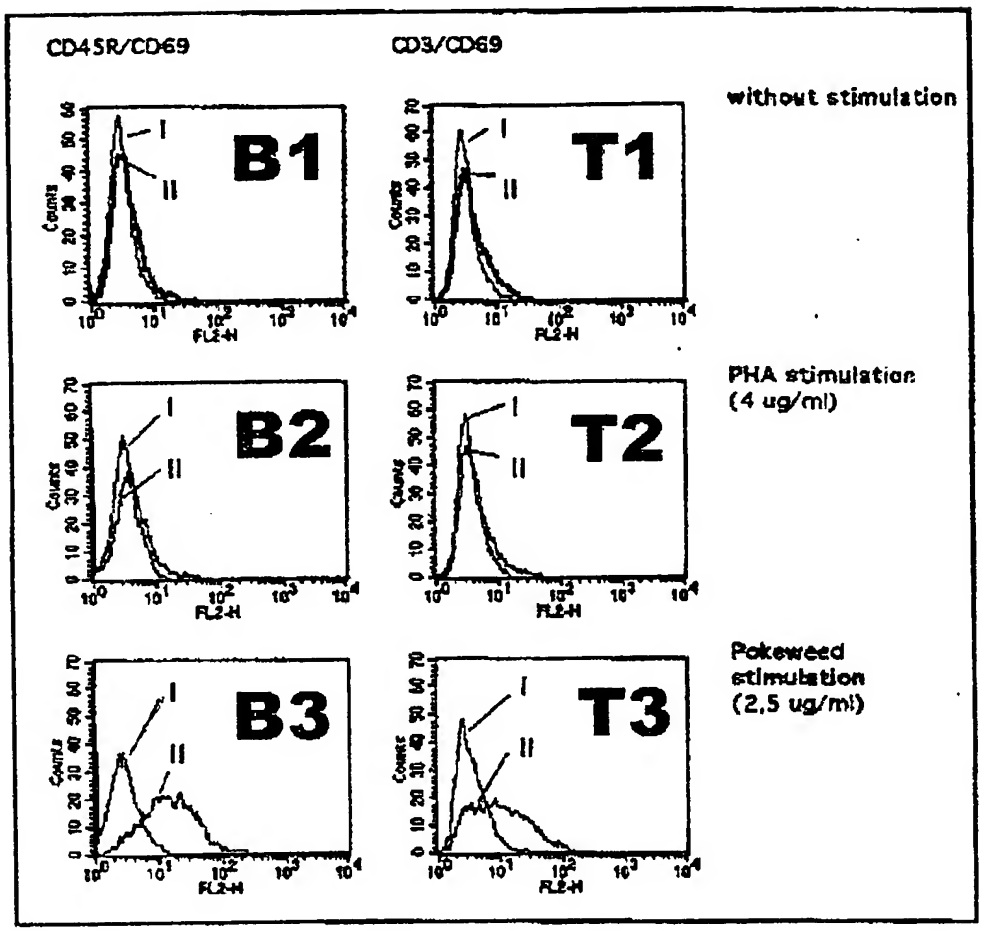
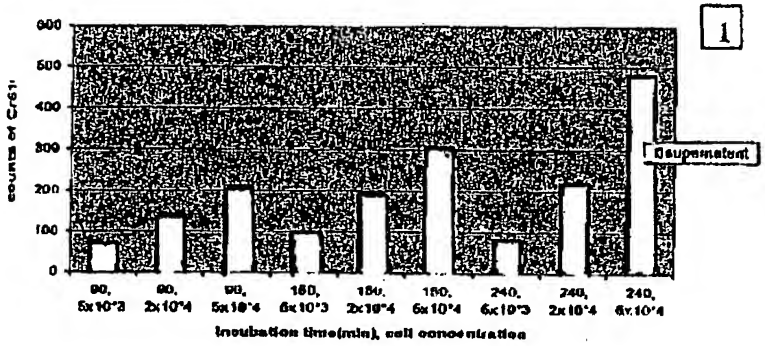


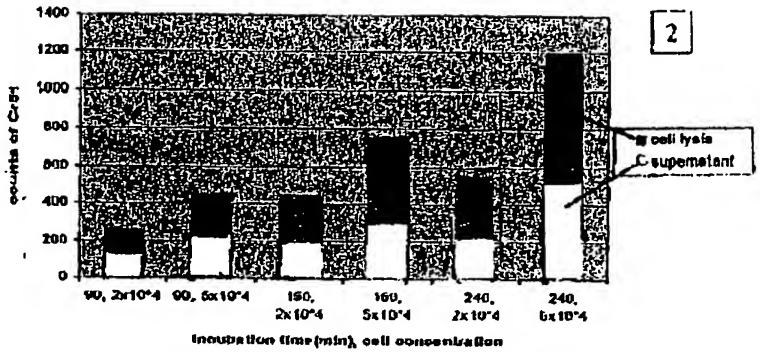
Fig. 13:

Representative FACS profiles of expression of various adhesion molecules on spleen cells from a female BALB/c mouse. B- and T cells were activated for three days with 8 g/ml PHA (Lectin from Phaesolus vulgaris Sigma, Cat.No.L-8754) (Fig. T2 and B2) or 2.5 g/ml Pokeweed (Lection from Phytolacca Americana, Sigma Cat.No.L-9379) (Fig.T3 and B3) and only FACS buffer (PBS, 2% FCS, 0,01% NaN_3) as negative control (Fig.T1 and B1) respectively. B cells were stained for single fluorescence with anti-mouse CD45R/ B220 (diluted 1:100 FACS buffer, RA3-6B2, PharMingen Cat.No.01124A, FITC) (Fig. B1, B2 and B3, lane I) and for double fluorescence with anti-mouse CD69 (diluted 1:100 in FACS buffer, H1.2F3, PharMingen Cat.No01505B, PE) and anti-mouse CD45R/ 220 (Fig.B1, B2, and B3, lane II, showing activated B cells.) T cells were stained for single fluorescence anti-mouse CD3 (diluted 1:100 in FACS buffer, 145-2C11, PharMingen Cat.No.01088A, Cy-Chrome) (Fig.T1, T2, and T3, lane I) and for double fluorescence with anti-mouse CD69 (diluted 1:100 in FACS buffer, H1.2F3, PharMingen Cat.No.01505B, PE) and anti-mouse CD3. Cells were sorted and measured in FACScan (Becton Dickinson) (Fig.T1, T2, and T3, lane II, showing the activated T cells).

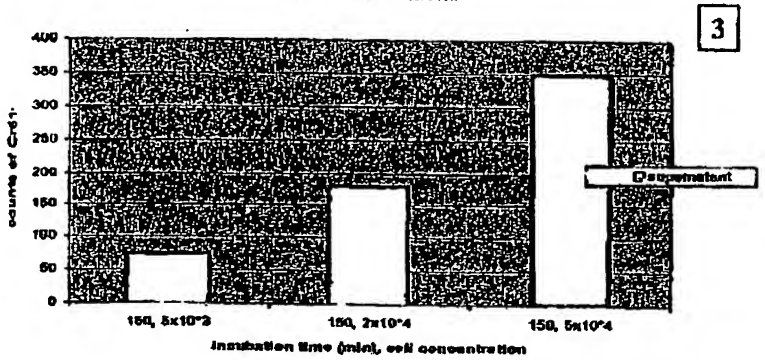
Titrationsexperiment with 100 µCi Cr51/10^7 cells and different cell concentrations and incubation time



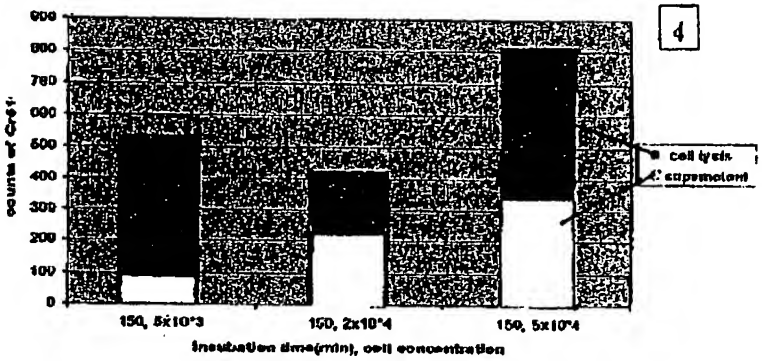
Titrationsexperiment with 100 µCi Cr51/10^7 cells



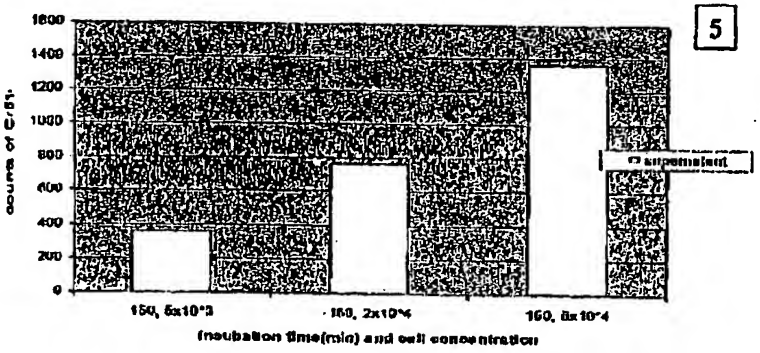
Titrationsexperiment with 200 µCi Cr51 / 10^7 cells and different cell concentrations



Titration-experiment with 200 µCi Cr51 / 10^7 cells



Titrationsexperiment with 400 µCi Cr51 / 10^7 cells and different cell concentrations



Titrationsexperiment with 400 µCi Cr51 / 10^7 cells

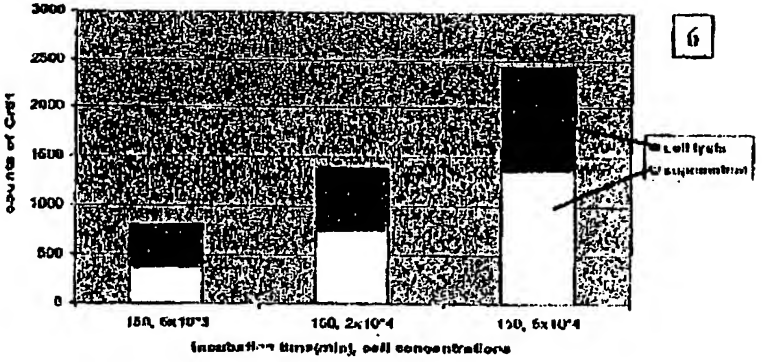
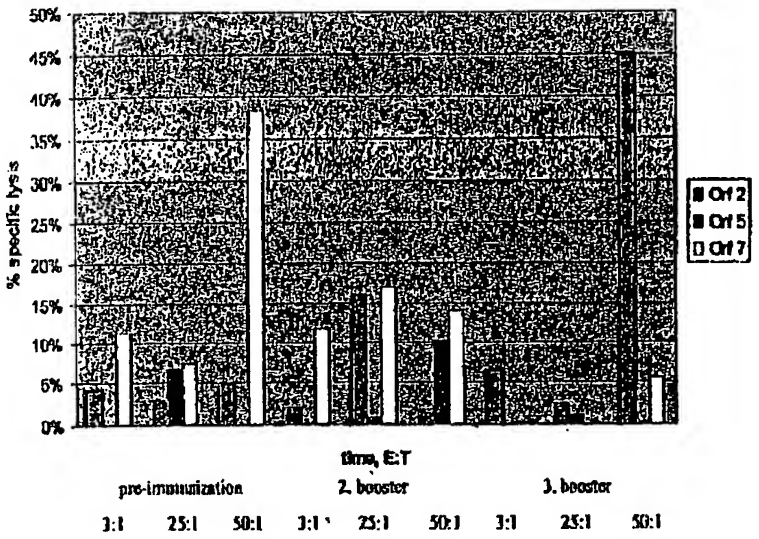


Fig. 14 a)



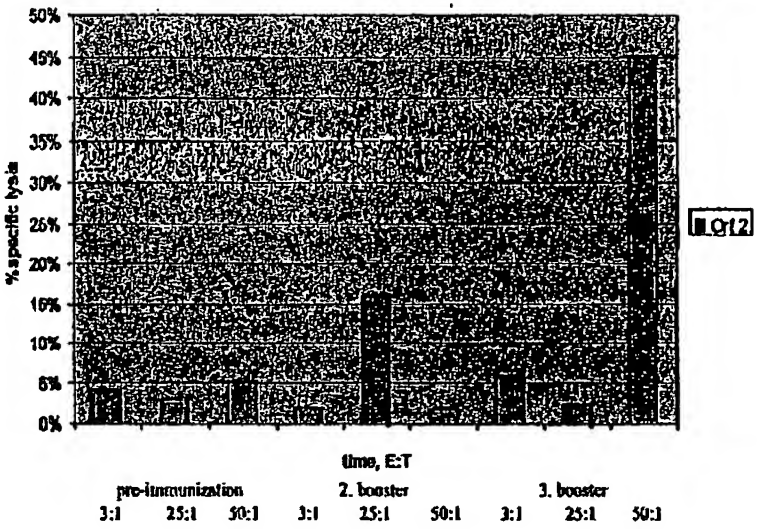
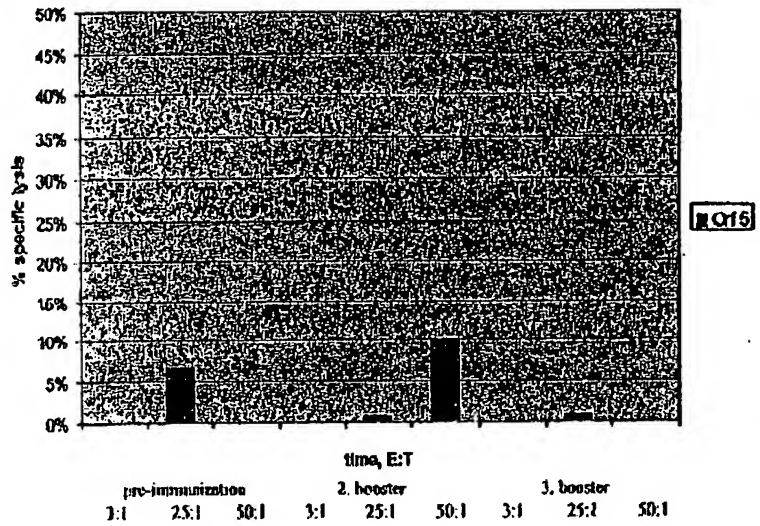
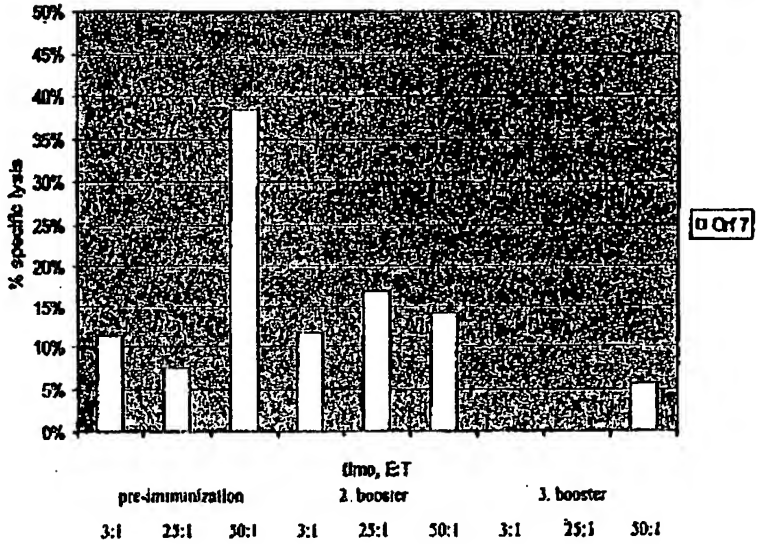


Fig. 15 c)





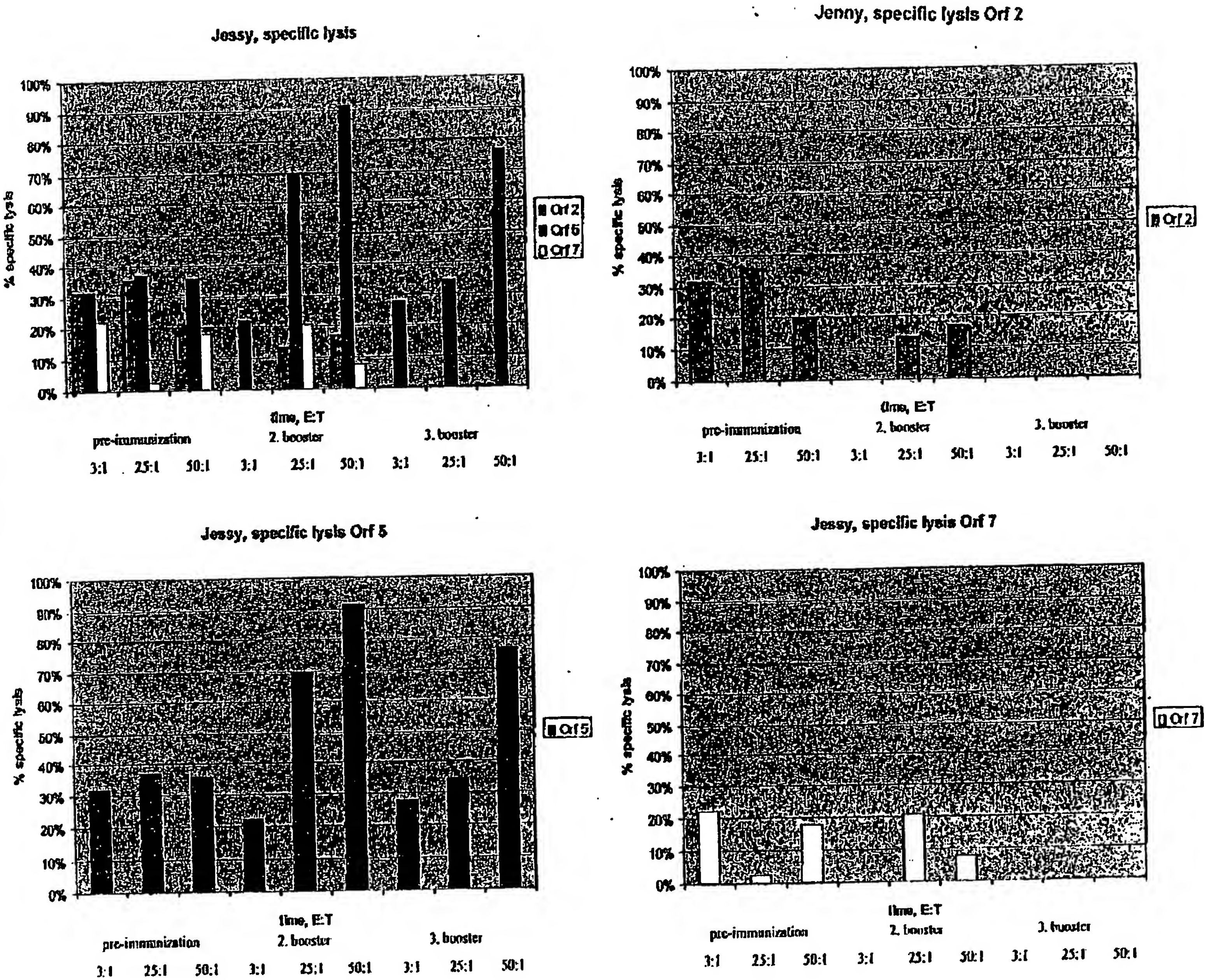

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Fig. 15 d)